Chapter 2

Development of Novel Repellents Using Structure−Activity Modeling of Compounds in the USDA Archival Database

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The United States Department of Agriculture (USDA) has developed repellents and insecticides for the U.S. military since 1942. Repellency and toxicity data for over 30,000 compounds are contained within the USDA archive. Repellency data from subsets of similarly structured compounds were used to develop artificial neural network (ANN) models to predict new compounds for testing. Compounds were then synthesized and evaluated for their repellency against *Aedes aegypti* mosquitoes. Rellency data, *i.e.*, complete protection time (CPT) were used to develop Quantitative Structure Activity Relationship (QSAR) models to predict repellency. Successful prediction of novel acylpiperidine structures by ANN models resulted in the discovery of compounds that provided protection more than three times longer than DEET. The acylpiperidine QSAR models employed 4 descriptors to describe the relationship between structure and repellent duration. The ANN model of the carboxamides did not predict compound structures with exceptional CPTs as accurately; however, several carboxamide candidates did perform as good as or better than DEET.

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History of the USDA Evaluation Program

In March 1942, funds from the National Emergency Council, Office of Scientific Research and Development (NEC-OSRD) were made available to the Bureau of Entomology and Plant Quarantine, Division of Insects Affecting Man and Animals, of the United States Department of Agriculture (USDA)-Agricultural Research Administration to expand the small field laboratory in Orlando, FL. Willard V. King was appointed to oversee the development of the Orlando laboratory. By the time the laboratory was fully operational, W.E. Dove had assumed the role of director, and he was followed by Ed Knipling in July, 1942 (*1*). The mission of the laboratory was to discover new chemicals and methods for the control of medically-important arthropod pests of the U.S. Armed Forces (*1–3*). Most of the early submissions received for screening by the Orlando laboratory for screening consisted of known commercially available insecticides and repellents either submitted by commercial entities, the Bureau of Entomology Insecticide Investigations, or by other agencies of the US Government as part of the OSRD. The program was expanded in June, 1944 to include Columbia, Harvard, Ohio State, and Stanford Universities, along with the Universities of Illinois, Maryland, Minnesota, and Wisconsin to provide candidate compounds for evaluation at the Orlando laboratory (*2*). On November 1, 1945, the source of funding for the program was changed to the U.S. Army Office of the Surgeon General, and in the late 1950s this funding line was transferred to the Insects Affecting Man and Animals Branch of the USDA-Entomology Research Division with the expectation that the program would continue the development of control methods to protect US service personnel from arthropod attack. The Orlando laboratory has changed names and locations throughout the years. In 1951, it was renamed the "Insects Affecting Man and Animals Research Laboratory (IAMARL), along with the formation of the "Mosquito Research Unit." The laboratory was moved from Orlando, FL, to Gainesville, FL, in 1962. The unit conducting mosquito research was renamed the "Mosquito and Fly Research Unit" in 1988. The laboratory was renamed the Medical and Veterinary Entomology Research Laboratory (MAVERL) in 1990, and finally the Center for Medical, Agricultural, and Veterinary Entomology (CMAVE) in 1996.

Early History of the USDA Repellent and Insecticide Program and Archive

On March 11, 1942, the Orlando laboratory of the Bureau of Entomology recorded its first chemical submission and using the code O-1 (Orlando-1). The sample consisted of six 10-oz jars of "Pyrinate" from McKesson & Robbins. Most of the submissions received over the next two months were pyrethrin mixtures and chlorinated hydrocarbons. The well known insecticide 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (DDT) was submitted as the active ingredient in two products named "Gesarol." The two products, O-1151 (dust) and O-1152 (spray), were logged into one of the archival record books on November 16, 1942, with information that the sample was received from the New York division of the J.R. Geigy Company (*3*).

Some of the best repellent active ingredients during this time period were Indalone (O-9), tested on March 27, dimethyl phthalate (O-262), tested on May 8, and Rutgers 612 (O-375), tested on June 15, 1942. These three repellents were mentioned by Ed Knipling as the best from the initial screening phase and they were recommended for U.S. Military use since they provided about 2 h protection time (*3*). However, military personnel still needed a repellent that would last for 10 hours. This need was met about a decade later when the most successful mosquito repellent to date, N,N-diethyl-3-methylbenzamide (DEET; Figure 1) was recorded as O-20218 on February 5, 1952. It had been sent from S.A.Hall, a chemist with the division of insecticide investigations, Bureau of Entomology and Plant Quarantine, at Beltsville, MD, to the Orlando laboratory and received by William C.McDuffie, assistant leader of the Insects Affecting Man and Animals Section of Entomology Research Branch. DEET was first screened as a clothing treatment and found to be a superior candidate (*4*). This led to its selection for field trials conducted in Panama in early 1953 (*5*). A second submission for DEET was recorded as O-22542 on December 17, 1953 and the following is written in the notebook:

> *"Reaction product of mixed toluic acid isomers (containing approx.*
> *70% m-toluic acid and 30% p-toluic acid) and diethylamine. Insecticide*
> *Investigations, Memo S.A. Hall to W.C.M. December 14, 1953, 50g."*

Under the USDA archival record system, the final compound submitted by the Beltsville laboratory was recorded in the notebook as AI3-55208 (formerly Orlando numbers, now AI3- numbers for "Agricultural Insecticide 3-"). It was sent by Al DeMilo of the Beltsville Laboratory on May 22, 1997, and tested by Don Barnard and his group in Gainesville on September 8, 1997. In actuality, sublots of formerly tested compounds continued to be received from Beltsville. The final recorded entry is for AI3-37220-Gf on May 12, 1998 and this compound will be discussed further later in this chapter.
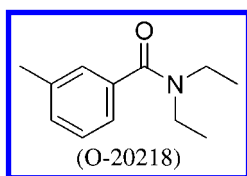

(O-20218)

Figure 1. Orlando number and structure of N,N-diethyl-3-methylbenzamide (DEET).

Recent Research of the USDA Insecticide and Repellent Program

Research by the USDA for the U.S. Military continued with lower intensity through the 1990s. A significant stimulus to reinvigorate the association between the USDA-Agricultural Research Service (ARS) and the U.S. Military was made possible in 2004 by a new Department of Defense funding line named the "Deployed War-Fighter Protection Program" (DWFP). The emphasis of the research program is on the development of novel or improved pesticide

chemicals and formulations, application technologies, and personal protection. It is within the realm of personal protection that the research and development of novel topical repellents is being conducted. There are several sources that provide repellents as part of this renewed collaborative effort. Among these are the USDA-ARS laboratories in Beltsville, MD, (Invasive Insect Biocontrol and Behavior Laboratory), Gainesville, FL (CMAVE) and Oxford, MS (Natural Products Utilization Research Unit), researchers at the University of Mississippi, and in Australia, French Polynesia, Germany, Isreal, Malaysia, Samoa, Saudi Arabia, and Turkey. While one objective of the laboratory in Gainesville, FL, is to provide insecticide and repellent evaluation for the DWFP, researchers at this laboratory are also devoted to the development of new repellents using the data contained in the historical archive. Through the use of modern methods of structure−activity modeling, the goals are: a) to understand better how chemical structure relates to repellency by developing accurate models and b) to develop improved repellents as an outcome of these models. This work involves collaboration with chemists at the University of Florida and the results of this effort are the subject of this chapter.

Structure−Activity and Computer Modeling

The examination of molecular structures and modeling can be traced back to the early 1900s (*6*). Prior to the development of computers, the examination of a set of chemicals and attempts to relate their activity to the structures was almost entirely dependent on the skill of the synthetic chemist to devise and synthesize structurally-similar compounds once a lead compound was identified. Upon examination of the archive, it is evident that the discovery of the repellent DEET was due to a process where related structures had been evaluated and found to be repellents. In the spring of 1952, the compounds *N,N*-diethylbenzamide (O-1197-d) and *o*-chloro-*N,N*-diethylbenzamide (O-17586-b) (Figure 2) were tested on skin against *Aedes aegypti*, with the latter compound protecting about 10% longer than the former (*4*). The compound *N,N*-diethylbenzamide had been received from the USDA Beltsville Insecticide Division and logged in originally on November 23, 1942, during the first year of the program. The O-17586-b compound noted above was received from Geigy Co.; however, this compound was originally received from the Insecticide Division and tested on March 29, 1946 (originally recorded as O-11147). Samuel Gertler applied for a patent covering the *N,N*-diethylbenzamides as repellents on September 4, 1944 and the patent was granted on Oct. 1, 1946 (*7*). Unfortunately, these compounds mentioned above produced skin irritation, so further repellent studies with them were abandoned. The continued efforts to produce structurally-similar substituted *N,N*-diethylbenzamides by the Beltsville chemists led to the discovery of DEET as one of the best repellents as noted in McCabe et al. (*8*).
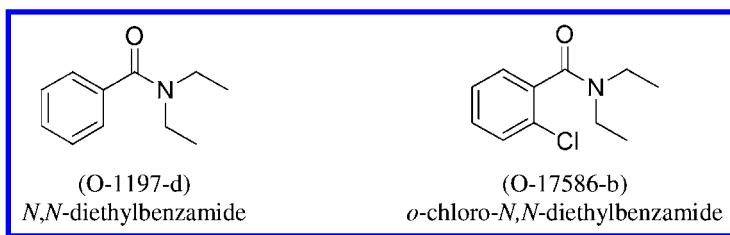
(O-1197-d)
N,N-diethylbenzamide

(O-17586-b)
o-chloro-*N,N*-diethylbenzamide

Figure 2. Repellents tested prior to the discovery of DEET.

Uses of Computer Modeling of Chemical Structures

Extensive use of computers in modeling began in the 1950s (*9*). The specifics of linear modeling of biological properties, specifically Quantitative Structure−Activity Relationships (QSAR), can be traced back to the work of Corwin Hansch and colleagues in the early 1960s (*10*). As noted by Hansch, contrary to the belief that the history and success of QSAR lies in the pharmaceutical domain, the earliest applications and successes involved the modeling of pesticides.

Application of Modeling Methods to Repellent Discovery

Structure−activity modeling has also been applied to repellent discovery, with perhaps one of the greater successes being the discovery of 2-(2-hydroxyethyl)-1-piperidinecarboxylic acid 1-methylpropyl ester, more commonly known by the names Picaridin, Icaridin, KBR 3023, or Bayrepel ® (Figure 3). This compound was discovered through structure−activity work in the 1980s (*11*) and tested as AI3-65545 on October 31, 1993.
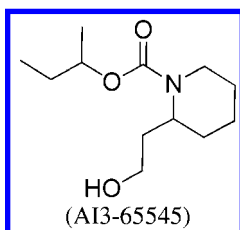

(AI3-65545)

Figure 3. Structure of Picaridin (KBR 3023)

Researchers have used 3D-QSAR of DEET and related analogues to construct pharmacophores to better understand the structural basis that leads to repellency by these amide compounds (*12–14*). Their model was constructed primarily from the protection time data of Suryanarayana et al. (*15*). Ma et al. (*12*) demonstrated that one could predict repellent duration based on compound structure, and specifically that the amide group and attached substituents played a significant role in the experimentally determined repellent efficacy. Using the same data set, Katritzky

In Recent Developments in Invertebrate Repellents; Paluch, G., et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2011.

et al. (*16*) applied Codessa Pro software (*17*) to develop a QSAR model for the prediction of complete protection time (CPT) from descriptors related to the structural and electronic properties of the DEET analogues. This work was the foundation for current projects that involve examination of repellency and toxicity data for subsets of compounds within the USDA Archive.

However, there is a weakness in the way that repellency data are recorded in the USDA archive and this impacts the development of structure−activity models. Instead of being reported in days or time of CPT, the repellent protection times were converted to a 5 class system based on CPT as detailed in Table I. The groupings are not only non-linear but tend to equate all superior repellents (class 5) as identical to one another when in fact there can be significant differences in numbers of days that compounds are repellent.

Table I. Five class system of repellents based on complete protection time (CPT) from treated cloth and stockings. SOURCE: Reproduced from reference (*18*). Copyright 2010 Entomological Society of America

Class	Minimum Day	Maximum Day
1	0	1
2	1	5
3	5	10
4	10	21
5	21	−

Fortunately, artificial neural networks (ANNs) can overcome these limitations and can be used to develop models for these types of data. Some of the earliest work with neural networks was that of McCulloch and Pitts in 1943 (*19*). They can be used for evaluation of non-linear data for the development of a predictive model. Thus, a non-linear data set, such as the class system of CPT data in the USDA archive, can be used to develop a model and predict compound activities based on the compound structures and associated repellent activities that were incorporated into the neural network.

Three-layer neural networks with different architectures were applied to the two data sets discussed in this chapter, *i.e*,. acylpiperidines and carboxamides.

Development of the ANN model was the first step used to predict new repellents. This was accomplished by selecting a set of similarly structured compounds from the USDA archive, then randomly dividing the compounds into a training set and a validation set. The training set contained approximately 75% of the compounds used to develop the model. The remaining compounds were then used as the validation set to verify the accuracy of the model. If there was good correlation between predicted values (classes in the case of repellents) and the experimentally determined class, then the ANN was used to predict classes

for compound structures that are input into the model. Some predicted structures were synthesized, and then evaluated for repellent efficacy by measurement of CPT, and in the case of the carboxamides, by both CPT and the minimum effective dosage (MED), which is the concentration required to produce repellency. Rather than converting these data to classes as had been done historically, the actual number of days of protection, or the threshold concentration of protection was used in efforts to develop QSAR models.

Measurement of Repellent Efficacy

Screening for Repellency of Compounds with Unknown Toxicology

In screening studies, approximately 500 colony-reared female *Ae. aegypti* (Orlando strain, 1952), aged 5-10 days and maintained on 10% sugar solution, were used per cage (approximately 46 cm x 36 cm x 36 cm ≈ 59,000 cm³). Since stock cages of mosquitoes contain both males and females, a drawbox was used to preselect females that responded to human odors with the appropriate host-seeking behavior (*20*).

Because the experimental compounds screened in these studies have unknown toxicology, they should not be applied directly to the skin. However, muslin cloth can be treated with the candidate as a means to test the compound without topical application (*21*). Compounds are placed in separate vials and dissolved into a solvent that evaporates rapidly, *e.g.* acetone. A 5 cm x 10 cm segment of muslin cloth is then added to the vial containing the compound in solution. The cloth is removed and dried until the solvent evaporates. When ready to be tested, a volunteer can affix the treated cloth to cover a 32 cm² opening on a specially designed vinyl sleeve (Figure 4). The hand of the volunteer is gloved to protect from bites, and the only accessible area for mosquitoes to bite is through the opening in the sleeve. The cloth does not come in direct contact with the skin because of a stocking worn underneath the sleeve to provide a small barrier between the cloth and skin. The use of skin emanations is needed to attract mosquitoes to the opening in the vinyl sleeve. However, just as with other laboratory-based screening methods, the performance of a compound on cloth only partially reflects what the performance would be like if applied directly on skin.

Since these studies involved human volunteers, all participants were required to provide informed consent to participate. All data were collected in accordance with the approved University of Florida Institutional Review Board (UF IRB) Project entitled, "Laboratory Evaluation of Repellents for Personal Protection from Mosquitoes and Biting Flies" (Project # 636-05).

Figure 4. Photo Credit: Greg Allen, USDA-ARS. Reproduced from reference (18). Copyright 2010 Entomological Society of America. (see color insert)

Duration of Repellent Efficacy

The repellency duration is measured by the complete protection time (CPT), which is the amount of time in days that a compound will fully protect the wearer from bites of a test population of mosquitoes or other biting arthropods. In the case of mosquitoes, the end point is normally measured as the "time to first bite," however, quite often a second bite is used to provide the "time to the first confirmed bite" (*22*). There are concerns about significant errors resulting from measurement of a single bite as an end point despite the CPT being a useful and understandable metric to compare repellent efficacies. Therefore, the end point is normally selected to be a threshold number of bites. In the experiments described here, the failure threshold was predetermined to be the point at which 1% of mosquitoes had bit through the cloth (5 bites out of the 500 mosquitoes in the cage) during the 1 min test period. The CPTs were determined at 25 µmol/cm^2 and 2.5 µmol/cm^2 concentrations (*18*, *23*). These concentrations were selected to bracket the amount of DEET that is typically applied directly to skin in repellency assays.

Threshold Concentration for Repellency

The threshold amount of a repellent needed to prevent bites is estimated by measuring the minimum effective dosage (MED) of the repellent (*18*)(*21*). A range of concentrations on cloth was used in these experiments starting with a high concentration of 25 µmol/cm^2. Serial dilutions were made from 25 µmol/cm^2 down to 3.125 µmol/cm^2 using the higher concentration solution, and from 2.5 µmol/cm^2 down to 0.020 µmol/cm^2 using the lower concentration solution. Similar to tests for CPT, the arm with treated cloth was inserted into the mosquito

cage and tested for 1 min. If < 5 bites are received (1% out of 500), then the compound is considered repellent at that concentration.

Acylpiperidine Repellents

Acylpiperidine repellents have been studied for decades. Picaridin (Figure 3), the active ingredient in a number of commercial products, belongs to this class of compounds. Two of the more efficacious experimental repellents discovered by the USDA Beltsville laboratory are also in this class: 1-(cyclohex-3-en-1-ylcarbonyl)piperidine (AI3-35765) and 1-(cyclohex-3-en-1-ylcarbonyl)-2-methylpiperidine (AI3-37220) (Figure 5).

(AI3-35765)
cyclohex-3-en-1-ylcarbonyl)piperidine

(AI3-37220)
1-(cyclohex-3-en-1-ylcarbonyl)-2-methylpiperidine
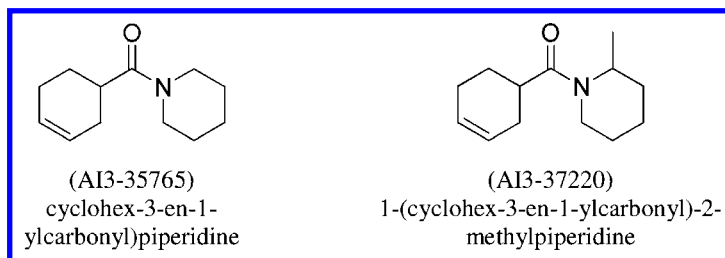
Figure 5. Piperidine repellents developed the USDA in the 1970s.

The AI3-35765 compound was tested on April 17, 1973, having been sent from the Organic Chemistry Synthesis Laboratory (OCSL) of the USDA Beltsville laboratory. On April 26, 1977, AI3-37220 was tested after it was synthesized by Terry McGovern of the OCSL (*24*). Later 3D-QSAR studies on Picaridin and 1-(cyclohex-3-ene-1-ylcarbonyl)-2-methylpiperidine (AI3-37220) using a hierarchical molecular overlay approach showed the importance of shape and molecular surface structure for effective repellent activity in the diastereoisomeric compounds of AI3-37220 (*25*). Calculations for the most active diastereoisomer (220SS) identified by Klun et al. (*26*) indicated a strong relationship between the structure and the biological potency.

Artificial Neural Network Modeling

The initial repellent model for the acylpiperidine data set was developed using 150 out of 200 selected acylpiperidines as the training set for the ANN. A full listing of the compounds, (coded by AI3- numbers), structural information, and notation of whether they were in the training or validation subsets can be found in the Supporting Information for Katritzky et al. (*23*). This set did not include AI3-35765 or AI3-37220 in the model, but did contain some compounds similar to those in structure (see Table II, *e.g.* **4a′-4d′** and others). The archival data used for the initial models in this study were accumulated from compounds submitted as early as 1942 and as late as 1994; the compound structures with AI3- numbers can be found in Table S1of the supplementary information of Katritzky et al. (*23*). Some of the modeled compounds were from acylpiperidines patented as insect repellents in 1981 (*27*).

The models for the acylpiperidines were developed with an 8-7-1 architecture, comprised of 8 initial descriptors as neurons for the input layer, followed by 7 neurons in a hidden layer, and the output of the predicted class as the final neuron. The input descriptors used to produce the best model were: 1) 3rd order Kier and Hall index, 2) molecular weight, 3) molecular surface area, 4) total molecular dipole moment, 5) total molecular electrostatic interaction, 6) total number of bonds in the molecule, 7) carbon atom surface area, and 8) nitrogen atom surface area. The resultant ANN model was able to predict the most efficacious repellents (class 4 and 5) with 71% accuracy (*23*).

With a satisfactory ANN model, structures can be devised and tested in the model to predict their repellent classes. This was performed with just over 2000 acylpiperidine structures. Some of these compounds had been tested previously, but many others were novel in the sense that they had not been evaluated previously as mosquito repellents. From 2000 predicted compounds, 34 were selected for synthesis: 23 were novel compounds, and 11 were chosen from those in the USDA archive. Selection of compounds that had been tested previously allowed for comparison and validation of the current repellent testing methodology with that used decades ago. The repellency data generated for this study were more precise and linear, *i.e.*, the repellency was measured in days of protection, rather than put into classes with non-linear distributions of protection time. Also, bioassays were conducted with stoichiometrically equivalent amounts of compounds, rather than comparison of gravimetrically equivalent amounts, as had been done historically. Generating data based on these changes was necessary for development of accurate QSAR models.

Synthesis

The selected 34 acylpiperidine mosquito repellent candidates **4a-q′** were synthesized according to the pathway of Figure 6 (*23*). Treatment of the carboxylic acids **1** with thionyl chloride and benzotriazole at 25 °C in methylene chloride in a 1:1:3 mole ratio produced 1-acylbenzotriazoles **2** (*23*). Reaction of 1-acylbenzotriazoles **2** with one equivalent of piperidines **3** in tetrahydrofuran, THF at 25 °C or in toluene under reflux resulted in formation of N-acylpiperidines **4a-q′**(Table II) in 71–100% yields using a procedure modified slightly from one used historically (*28*).
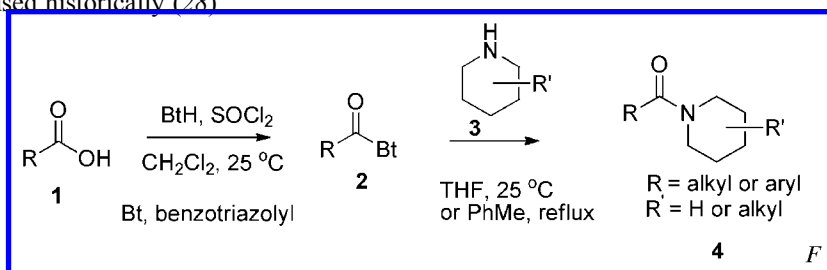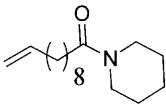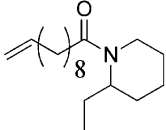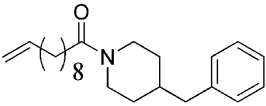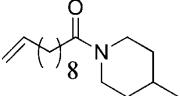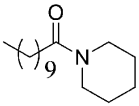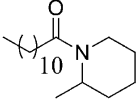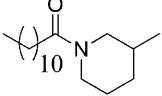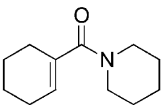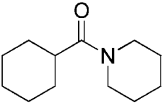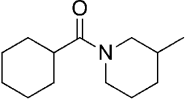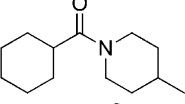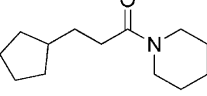

*Figure 6. Preparation of acylpiperidines **4**. Reproduced from reference (23). Copyright 2008 The National Academy of Sciences of the USA.*

Table II. Compounds used for the acylpiperidine repellent study

ID	Name	Structure
DEET	N,N-diethyl-3-methylbenzamide (O-20218)	
4a[a]	1-acetyl -2-methylpiperidine	
4b[a]	1-(1-oxopropyl)-piperidine	
4c[a]	2-ethyl-1-(1-oxopropyl)-piperidine	
4d[a]	2-methyl-1-(1-oxoheptyl)-piperidine	
4e[a]	3-methyl-1-(1-oxoheptyl)piperidine	
4f[a]	4-methyl-1-(1-oxooctyl)piperidine	
4g[a]	1-(1-oxooctyl)-4-(phenylmethyl)piperidine	
4h[a]	2-ethyl-1-(1-oxononyl)piperidine	
4i[a]	2-methyl-1-(1-oxodecyl)piperidine	
4j[a]	4-methyl-1-(1-oxodecyl)piperidine	

Table II. (Continued). Compounds used for the acylpiperidine repellent study

4k	1-(1-oxo-10-undecylenyl)piperidine AI3-39049	
4l	2-ethyl-1-(1-oxo-10-undecylenyl)piperidine	
4m[a]	1-(1-oxo-10-undecylenyl)-4-(phenylmethyl)piperidine	
4n[a]	4-methyl-1-(1-oxo-10-undecylenyl)piperidine	
4o[a]	1-(1-oxoundecyl)piperidine	
4p[a]	2-methyl-1-(1-oxododecyl)piperidine	
4q[a]	3-methyl-1-(1-oxododecanyl)piperidine	
4a'	1-(1-cyclohexen-1-ylcarbonyl)piperidine (AI3-38739)	
4b'	1-(cyclohexylcarbonyl)piperidine (AI3-36324)	
4c'	1-(cyclohexylcarbonyl)-3-methylpiperidine (AI3-36537)	
4d'	1-(cyclohexylcarbonyl)-4-methylpiperidine (AI3-36538)	
4e'	1-(3-cyclopentyl-1-oxopropyl)piperidine (AI3-38423)	

Continued on next page.

Table II. (Continued). Compounds used for the acylpiperidine repellent study

4f[a]	1-(1-methylcyclohexylcarbonyl)-3-methylpiperidine	
4g'	2-methyl-1-[(4-methylcyclohexyl)carbonyl]piperidine (AI3-39012)	
4h'	1-(cyclohexylcarbonyl)-2-ethylpiperididne (AI3-36539)	
4i'	1-(cyclohexylacetyl)-2-methylpiperidine (AI3-37409)	
4j'[a]	1-(3-cyclohexyl-1-oxopropyl)-2-methylpiperidine (AI3-37424)	
4k'	1-(3-cyclohexyl-1-oxopropyl)-3-methylpiperidine (AI3-37425)	
4l	1-(3-cyclohexyl-1-oxopropyl)-4-methylpiperidine	
4m'[a]	1-(4-cyclohexyl-1-oxobutyl)-4-methylpiperidine	
4n'[a]	1-(3-cyclopentyl-1-oxopropyl)-2-ethylpiperidine	
4o'[a]	1-(3-cyclohexyl-1-oxopropyl)-2-ethylpiperidine	
4p'[a]	1-(cyclohexylacetyl)-4-(phenylmethyl)piperidine	
4q'[a]	1-(3-cyclohexyl-1-oxopropyl)-4-(phenylmethyl)piperidine	

[a]Novel compounds

Bioassays of Compounds

The CPTs for the 34 acylpiperidines were determined at two selected concentrations (25 μmol/cm^2 and 2.5 μmol/cm^2). At the higher concentration, approximately one-third of the compounds were repellent on cloth for a duration that was greater than three times the repellent duration of DEET (Figure 7). The compound 4-methyl-1-(1-oxo-10-undecylenyl)piperidine (**4n**) prevented bites for an average of 73 days compared to 17.5 days for DEET at the 25 μmol/cm^2 concentration (Figure 7, Table II). This same compound lasted an average of 13 days compared to 2.5 days for DEET when tested at the lower concentration.
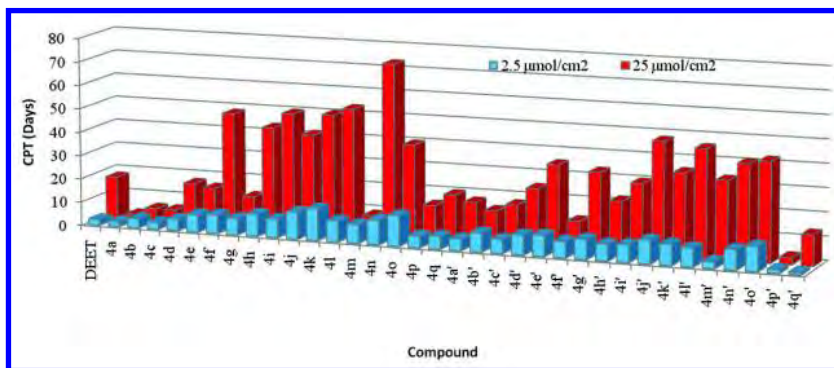
Figure 7. Complete protection time (CPT) of two concentrations of 23 novel and 11 previously tested acylpiperidines (see Table II for compound structures). (see color insert)

When the compounds that provided the greatest CPT are compared, there are noticeable similarities in their structures. Compound **4n** has a *para*- methyl on the piperidine ring with a 10-carbon terminally unsaturated chain as the acyl substituent. Very similar in structure to **4n** are **4k** and **4l**, which have the same acyl chain but no substituent on the piperidine ring (**4k**) and an *ortho*- ethyl on the piperidine ring (**4l**). Similarly, **4o** has a fully saturated 10-carbon acyl chain and again no substituent on the piperidine ring. Compounds **4i** and **4j** have 9-carbon fully saturated acyl chains with *ortho*- methyl on the piperidine for **4i** and a *para*- methyl on the piperidine for **4j**. Similar to **4j**, compound **4f** has a *para*-methyl on the piperidine ring, but instead has a 7-carbon saturated acyl chain. The cluster of compounds from **4j′-4o′** all have an acyl group consisting of a terminal cyclohexyl group or cyclopentyl in the case of **4n′**. The total number of carbons in the acyl group for each compound ranges from 7-9. The piperidine group either has a methyl substituent at the *ortho*-, *meta*-, or *para*- position, or has an *ortho*- ethyl group. Therefore, the general trend for acylpiperidines that last longer than DEET is that they: 1) contain no substituents, have monomethyl- or monoethyl- groups on the piperidine ring and 2) have an acyl group chain of 7-10 carbons, either saturated or terminally unsaturated, or having a terminal cyclopentane or cyclohexane. Presumably, the substituents reduce the volatility of these molecules and do not interfere with the structural properties that result in repellency when mosquitoes come in contact with these compounds.

If the repellency data for the 25 µmol/cm^2 and 2.5 µmol/cm^2 concentrations are converted to classes and plotted against the predicted class based on the ANN model, there appears to be little agreement between predicted and experimental classes for most of the compounds (Figure 8). In fact, the correlation between these data as predicted by ANN and the experimentally determined CPT converted to class is actually extremely low (R^2=0.007 and 0.006 for the high and low concentrations, respectively). Therefore classes are clearly not the best activity data to input for model development. Conversion back to classes results in non-linearity of the repellency data and reduces the number of "divisions" by which the repellent activity can be separated for the studied compounds.
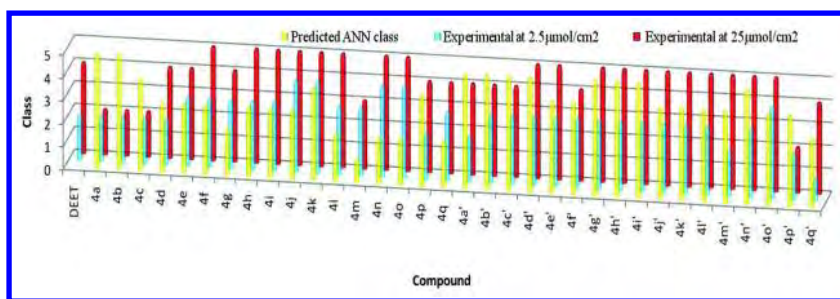
Figure 8. Comparison of predicted and experimental classes at two tested concentration levels of 25 and 2.5 µmol/cm^2 of 23 novel and 11 previously tested acylpiperidines.(see Table II for compound structures). (see color insert)

Maintaining activities as days of repellent duration is better for modeling purposes. Instead of an input if only 5 classes for the repellent activity, the range of activity can be input as the mean number of effective days of protection, from 1 to 73 days for the high concentration and from 0 to 13.5 days for the low concentration. Since each input was the mean duration of protection for two volunteers, this resulted in the possibility of half-day increments which effectively doubled the number of discrete values for the repellency activity at each concentration level.

Development of a QSAR Model

The results of bioassays (averaged days of CPT) were used to generate two QSAR models, one for the high (25 µmol/cm^2) and one for the low (2.5 µmol/cm^2) concentrations of compounds. Examination of the data distribution for each concentration revealed that the data acquired at the lower concentration had the more Gaussian distribution. In general, the more Gaussian the distribution of data used in a model, the more reliable the model is expected to be (*23*). The models were developed using 4 descriptors since adding additional descriptors complicated the model without adding a significant improvement in the predictive reliability (Table III).

**Table III. Best 4 descriptors models and their statistical parameters.
SOURCE: Reproduced from reference (*23*). Copyright 2008 The National
Academy of Sciences of the USA**

#	B^a	S^b	t^c	IC^d	Name of descriptor[e]
			25 μmol/cm²[f]		
0	-188.8	84.08	-2.246		Intercept
1	-2686	461.3	-5.823	0.09647	Maximum 1-electron reactivity index for atom C
2	-2616	488.2	-5.359	0.7253	Principal moment of inertia C
3	2.040	0.6920	2.948	0.3632	Maximum e-e repulsion for bond C-C
4	-0.02195	0.009215	-2.382	0.7759	WPSA-2 Weighted PPSA (PPSA2*TMSA/1000)
			2.5 μmol/cm²[g]		
0	-726.1	329.3	-2.205		Intercept
1	-68.13	9.393	-7.254	0.5248	YZ Shadow / YZ Rectangle
2	58.50	13.22	4.426	0.7120	Molecular volume/XYZ Box
3	-71.37	16.41	-4.350	0.5696	RNCG Relative negative charge (QMNEG/QTMINUS)
4	1.870	0.8053	2.321	0.2822	Minimum e-n attraction for bond C-O

[a] B, regression coefficient. [b] S, regression coefficient error. [c] *t*, Student criterion. [d] IC, partial intercorrelation. [e] PPSA, partial positively charged molecular surface area; WPSA, weighted PPSA; RNCG relative negative charge, ratio between the maximum atomic negative charge and sum of the negative atomic charges in the molecule. [f] $N = 4$; $n = 34$; $R^2 = 0.729$; $R^2_{cvOO} = 0.638$; $R^2_{cvMO} = 0.628$; $F = 19.50$; $s = 9.769$. [g] $N = 4$; $n = 34$; $R^2 = 0.689$; $R^2_{cvOO} = 0.608$; $R^2_{cvMO} = 0.582$; $F = 16.05$; $s = 1.815$.

Models for the high and low concentrations had good R^2 values (0.729 and 0.689, respectively) (Figure 9); however, it is obvious from Table III that the descriptors used to develop the models at each concentration were different. There are probably many reasons to explain these differences, but one of these is the difference in data distribution (normal or Gaussian), as noted earlier (*23*). Another reason may lie in the number of descriptors that the Codessa Pro software can employ in generating a model. Some of these descriptors may be similar to others and once the first is selected, other descriptors are chosen sequentially to be orthogonal to those already selected. An example of this similarity between non-identical descriptors can be seen in Table S5 from Katritzky et al. (*23*), where descriptor 3 (RNCG Relative Negative Charge) of the 2.5 μmol/cm² concentration model is highly intercorrelated with descriptors 2 (Principle Moment of Inertia C) and 4 (WPSA-2 weighted PPSA) of the 25 μmol/cm² concentration model at the 0.78 and 0.92 levels, respectively.

When the experimentally determined mean CPTs for the 25 μmol/cm² and the 2.5 μmol/cm² concentrations are compared to the QSAR model predicted values, it

is visually evident that there is close agreement for many of the compounds (Figure 10 for the 25 µmol/cm^2 and the Figure 11 for the 2.5 µmol/cm^2) concentrations.
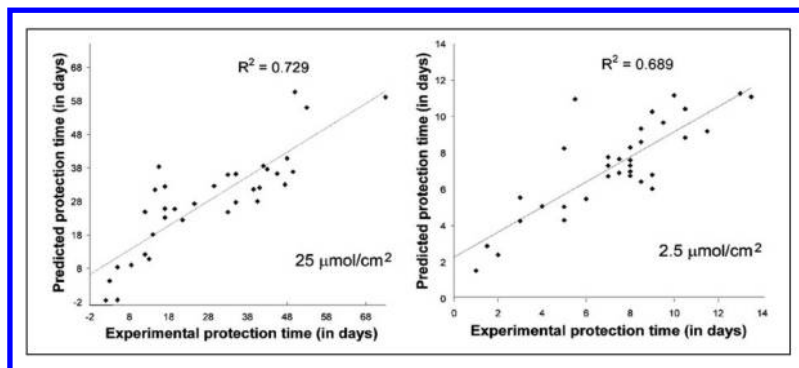

Figure 9. Comparison of predicted and experimental protection times for the two tested concentrations of acylpiperidines. Reproduced from reference (23). Copyright 2008 The National Academy of Sciences of the USA.
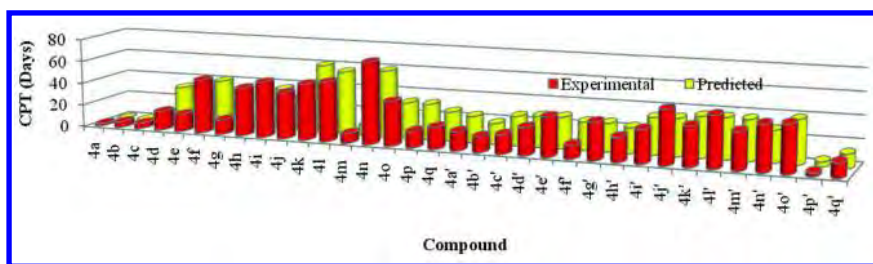

Figure 10. Comparison of experiment and predicted complete protection times(CPTs) for the high concentration (25 µmol/cm^2) of 23 novel and 11 previously tested acylpiperidines (see Table II for compound structures). (see color insert)
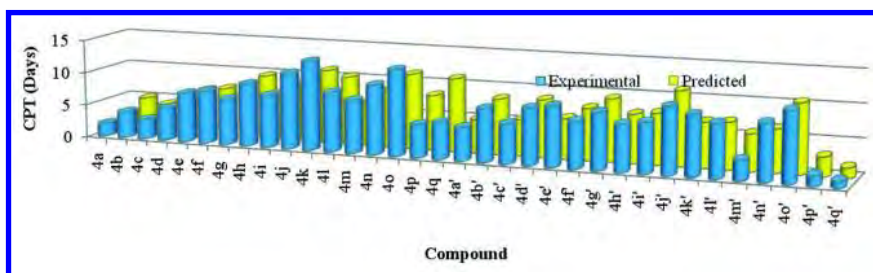
Figure 11. Comparison of experiment and predicted complete protection times(CPTs) for the low concentration (2.5 µmol/cm^2) of 23 novel and 11 previously tested acylpiperidines (see Table II for compound structures). (see color insert)

Carboxamide Repellents

Encouraged by the success of modeling acylpiperidines using the days of CPT as the measured biological parameter, a more structurally diverse set of carboxamides was selected for ANN modeling to predict novel carboxamide structures as candidate repellents (*18*). The data were selected from repellency classes of compounds submitted to the USDA and archived between November, 1952, and November, 1992.

Artificial Neural Network Modeling

As in the acylpiperidine model development, classes of repellency were used for the carboxamides ANN model. A total of 167 carboxamides were randomly divided into a 120-compound training set and a 47-compound validation set. Up to 1557 descriptors were calculated for each of the compounds. The carboxamide ANN model differed from the acylpiperidines in both architecture and descriptors used. The architecture of the carboxamides model consisted of 6 input neurons, followed by 4 hidden neurons, with the final output neuron as the repellency class. The descriptors used for the input neurons were: 1) weighted partial positive surface area based on Zefirov's partial charge, 2) average H atom valency, 3) molecular volume/XYZ box, 4) highest normal mode vibration frequency, 5) highest normal mode vibration transition dipole, and 6) minimal resonance energy for the C-H bond.

The model predicted the correct class for 70 of the 120 compounds in the training set, with 115 out of 120 predicted within one class ($R^2 = 0.622$). The class 4 and class 5 compounds were used to design 144 similar structures that were input into the carboxamide ANN model. Of the 144 of these that were input, 34 of the compounds predicted to be the highest classes were then synthesized. Based on the structure of these compounds, 4 additional compounds were synthesized for bioassay testing (Table IV).

Synthesis

The selected 38 carboxamides **5a-l′** were synthesized according to the scheme in Figure 12 (*18*). Treatment of the carboxylic acids **1** with thionyl chloride and benzotriazole in methylene chloride in a 1:1:3 mole ratio at 20 °C gave 1-acylbenzotriazoles **2** using a modified procedure (*29*). Reaction of 1-acylbenzotriazoles **2** with one equivalent of secondary amines **4** either in THF at 20 °C or in toluene under reflux gave carboxamides **5a-5u, 5j′** and **5k′** in 70–100% and **5i′** and **5l′** in 36 and 28% yields respectively (path A) (*30*). Path B was chosen for the preparation of the carboxamides **5v-h′** to avoid undesired Michael-type addition of benzotriazole to carboxamides **5** when non-blocked α,β-unsaturated 1-acylbenzotriazoles **2** are reacted with a secondary amines under neutral conditions. The resulting mixture of by-product Bt1-adduct **6b**, byproduct Bt2-adduct **6a** and the desired product **5x** could not be separated by column chromatography. Acid chlorides **3** were either commercially available or prepared in situ by treatment of the corresponding carboxylic acids **1** with 20–27% excess

of thionyl chloride at 20 °C overnight. Reaction of acid chlorides **3** with one equivalent of secondary amines in THF in the presence of 8% excess of sodium hydride at 0 to 20 °C led to formation of carboxamides **5v-h′** in 70–97% yield. The structures of the carboxamides **5a-l′** are given in Table IV.
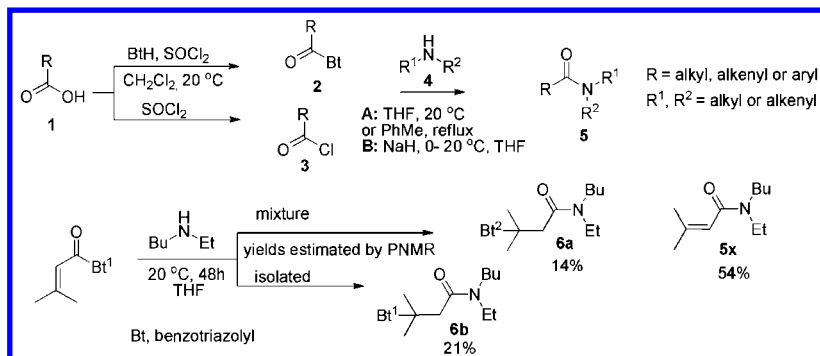

*Figure 12. Preparation of carboxamides **5**. Reproduced from reference (18). Copyright 2010 Entomological Society of America.*

Bioassays of Compounds

Although the ANN model adequately predicted classes for the compound structures used in the training and validation sets, when bioassayed most of the selected compounds were not as repellent as had been predicted. Possible reasons for this are that the diversity of the set and the non-linearity of the data prevented a successful correlation of predicted compounds with their experimentally determined efficacy. Over 50% of the compounds (23 out of 38) were predicted to be Class 4 and 5 (at least equivalent to DEET); however, only 11 of these had a CPT greater than that of DEET (Figure 13). At the 25 μmol/cm^2 concentration, the compound with the highest CPT (22 days), just over three times the duration of DEET, was a novel compound, (E)-*N*-cyclohexyl-*N*-ethyl-2-hexenamide (**5g′**) (Table IV). This compound lasted about twice as long as DEET when tested at the 2.5 μmol/cm^2 concentration.

Table IV. Compounds used for the carboxamide repellent study

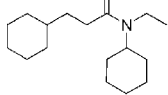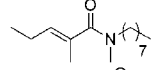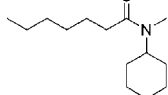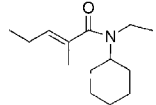| ID	Name	Structure
DEET	N,N-diethyl-3-methylbenzamide	
5a	N-butyl-N-methyl-hexanamide	
5b[a]	N-butyl-N-ethylhexanamide	
5c	N,N-diallylhexanamide	
5d	hexahydro-1-(1-oxohexyl)-1H-azepine	
5e	N-cyclohexyl-N-ethylhexanamide	
5f	N-ethyl-N-phenylhexanamide	
5g[a]	N-butyl-N-ethyl-2-methylpentanamide	
5h[a]	1-(1-azepanyl)-2-methyl-1-pentanone	
5i[a]	N-butyl-N,2-diethylbutanamide	
5j[a]	N,2-diethyl-N-(2-methyl-2-propenyl)butanamide	
5k[a]	N-butyl-N-ethyl-3-methylbutanamide	
5l	N,N-diisobutyl-3-methylbutanamide	
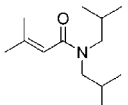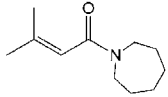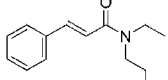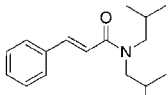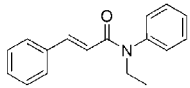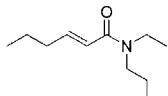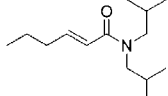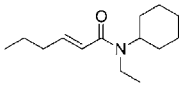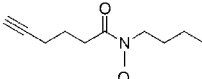
Continued on next page.

Table IV. (Continued). Compounds used for the carboxamide repellent study

5m[a]	N-cyclohexyl-N-ethyl-3-methylbutanamide	
5n[a]	N-butyl-N-ethyl-2,2-dimethylpropanamide	
5o[a]	N-ethyl-2,2-dimethyl-N-(2-methyl-2-propenyl)propanamide	
5p	1-(1-azepanyl)-2,2-dimethyl-1-propanone	
5q[a]	N-butyl-N-ethyl-2-methylbenzamide	
5r[a]	(E)-N-butyl-N-ethyl-2-methyl-2-pentenamide	
5s[a]	(E)-N-ethyl-2-methyl-N-(2-methyl-2-propenyl)-2-pentenamide	
5t[a]	(E)-1-(1-azepanyl)-2-methyl-2-penten-1-one	
5u	(E)-2-methyl-N,N-di-2-propenyl-2-pentenamide	
5v[a]	N-ethyl-2-methyl-N-(2-methyl-2-propenyl)benzamide	
5w	N-ethyl-2-methyl-N-phenyl-benzamide	
5x[a]	N-butyl-N-ethyl-3-methyl-2-butenamide	
5y[a]	N-ethyl-3-methyl-N-(2-methyl-2-propenyl)-2-butenamide	

Table IV. (Continued). Compounds used for the carboxamide repellent study

|		
5z	N,N-diisobutyl-3-methyl-crotonamide	
5a'	hexahydro-1-(3-methylcrotonoyl)-1H-azepine	
5b'	N-butyl-N-ethyl-cinnamamide	
5c'[a]	N,N-bis(2-methylpropyl)-3-phenyl-2-propenamide	
5d'	N-ethyl-N,3-diphenyl-2-propenamide	
5e'[a]	(E)-N-n-butyl-N-ethyl-2-hexenamide	
5f'[a]	(E)-N,N-di-(2-methylpropyl)-2-hexenamide	
5g'[a]	(E)-N-cyclohexyl-N-ethyl-2-hexenamide	
5h'[a]	N-butyl-N-methyl-5-hexynamide	
5i'[a]	N,3-dicyclohexyl-N-ethylpropanamide	
5j'[a]	(E)-N,2-dimethyl-N-octylpent-2-enamide	
5k'[a]	N-cyclohexyl-N-methylheptanamide	
5l'[a]	(E)-N-cyclohexyl-N-ethyl-2-methylpent-2-enamide	

[a]Novel compounds
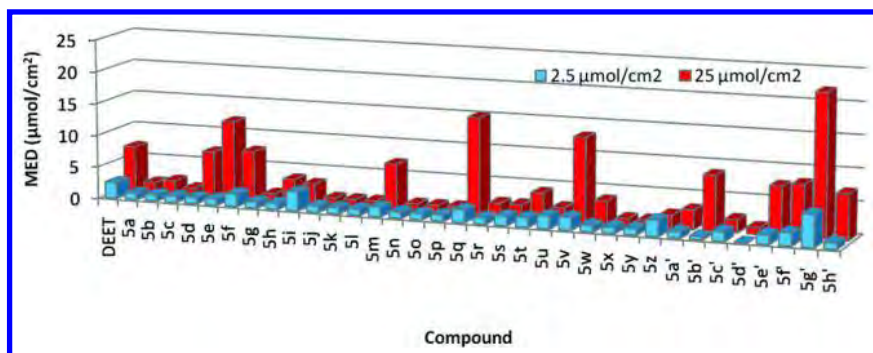
Figure 13. Complete protection time (CPT) of synthesized carboxamides compared to DEET at two concentrations (25 µmol/cm² and 2.5µmol/cm²). (see color insert)

Unlike the acylpiperidines, similarities among the best repellents (those with highest CPT) are not as apparent. Compound **5g′** has an ethyl- group and cyclohexyl group attached to the amide nitrogen, with a 5-carbon chain in the acyl group that is unsaturated next to the carbonyl. The same substituents on the *N*- and a fully saturated 5-carbon acyl chain results in **5e** having a CPT about two times longer than DEET. Compound **5q** had the second longest CPT and its structure is similar to DEET, having an *ortho* - methylbenzene attached to the carbonyl carbon, and ethyl and butyl groups attached to the nitrogen. Another of the better compounds, **5v**, is similar to **5q** on the acyl side, also contains an ethyl group attached to the amide nitrogen, but has a 2-methylpropene as the other substituent.

The non-linearity of the data and lack of widespread differences in repellent duration did not allow the development of QSAR models (*18*). Therefore, it was decided to examine the MED of the synthesized carboxamides. The compounds hexahydro-1-(1-oxohexyl)-1H-azepine (**5d**) had a MED that was equivalent to that of DEET (0.047 ± 0.007) µmol/cm² (Figure 14). Other compounds that were nearly equivalent in potency were (E)-1-(1-azepanyl)-2-methyl-2-penten-1-one (**5t**) at 0.098 ± 0.20 µmol/cm² and similarly structured 1-(1-azepanyl-)-2-methyl-1-pentanone (**5h**) at 0.102 ± 0.033 µmol/cm², followed by *N*-butyl-*N*-ethyl-2-methylpentanamide (**5g**) at 0.104 ± 0.16 µmol/cm². There was no apparent correlation noticeable between the most potent compounds having the lowest MED and compounds that were the least volatile (greatest CPT). However, it appears that the most potent repellents (those having the lowest MED) contain an azepine ring on the amide nitrogen. The compounds **5d**, **5h**, **5t** all have 5-carbon chains on the acyl side, with **5h** and **5t** having a methyl branch and **5t** with an unsaturated bond. The compound **5a′** has a relatively low MED and is similar to **5t** except that the unsaturated acyl group contains one less carbon. The least potent of this series is **5p**, containing a *t*-butyl group.
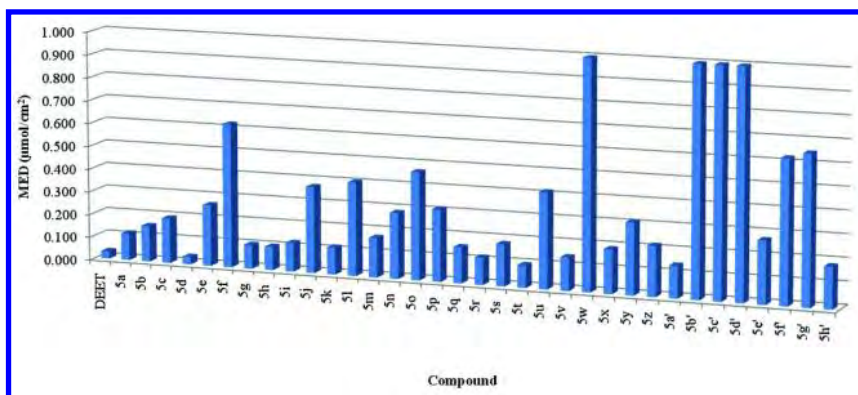
Figure 14. Minimum effective dosage (MED) of synthesized carboxamides compared to DEET. (see color insert)

Summary and Future Work

The repellency class data of a set of acylpiperidines from the USDA archive were used to develop suitable ANN models to predict new repellent structures. Predicted compounds that had not been previously examined for repellency along with compounds tested as repellents during the past 70 years were bioassayed for CPT. The results were used to develop a successful QSAR model to predict repellency duration (CPT) giving excellent correlation with experimental data. Some of these compounds had a duration of repellency three times better than DEET.

The approach used to produce the successful modeling and prediction of acylpiperidines was also applied to a subset of carboxamides. Perhaps due to the greater structural diversity, or imprecision in the non-linear class data, ANN models were not as successful in the prediction of repellents with high efficacy. However, despite the inability to produce a QSAR model of the carboxamide, about one-third of them had a CPT comparable or superior to DEET and another of the compounds had a MED equivalent to DEET.

Ongoing studies are being conducted to evaluate the acylpiperidines and carboxamides against other species, specifically ticks, and mosquito species that transmit malaria, such as *Anopheles gambiae and An. albimanus.* Traditionally, these mosquito species have been more difficult to repel than *Ae. aegypti.* Additionally, modeling approaches are being applied to mosquito and house fly adulticide and larvicide data found in the USDA archive.

Acknowledgments

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